

MOLECULAR CLONING AND EXPRESSION OF A cDNA ENCODING A PUTATIVE PEPTIDE RECEPTOR FROM THE RAT HYPOTHALAMUS. M.S. Statnick, N.G. Mayne, D.R. Gehlert, J.P. Burnett. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285-0424.

Neuropeptide Y (NPY) receptors represent a family of G-protein coupled receptors that have great molecular diversity. To gain further insight into the molecular structure of NPY receptors, we have attempted to clone new members of this receptor family. Using degenerate primers to conserved sequences of the neuropeptide Y1 receptor and the polymerase chain reaction (PCR), we amplified and cloned a cDNA fragment from rat genomic DNA. A PCR generated single-stranded ^{32}P -labeled probe of this cDNA was used to screen a rat hypothalamus cDNA library. Three clones were isolated and sequenced. Analysis of these sequences indicated an incomplete 5' coding sequence. Using asymmetric PCR in the rat hypothalamus cDNA library, we cloned the remaining 5' sequence and reconstructed the full coding sequence of the cDNA. The ~2.5 kb "composite" clone encodes a 306 amino acid protein that has seven hydrophobic domains. The cloned cDNA has highest homology to NPY, somatostatin and opioid receptors. Southern blot analysis of rat genomic DNA suggests that this receptor is encoded by a single gene. By Northern blot analysis a single ~6-kb transcript was identified in the rat brain. No mRNA was detected in any of the peripheral tissues examined. Analysis by RT-PCR showed that the receptor is expressed in many areas of the brain and pituitary. Using *in situ* hybridization, the highest levels of expression were localized to the anterior and intermediate lobes of the pituitary, reticular thalamic nucleus, paraventricular nucleus of the hypothalamus and granule cell layer of the cerebellum. Lower amounts of transcript were found in other brain areas. Following transient expression in AV12 cells, the receptor failed to bind NPY, somatostatin or opioid receptor ligands. Expression of an HSV-tagged version of the receptor in AV12 cells was detected in large amounts using fluorescence microscopy following transfection. Thus, it appears that cells transfected with this cDNA are capable of expressing the recombinant protein. We suggest that this cDNA encodes an orphan peptide receptor that may be involved in neuroendocrine regulation. The recent identification of an endogenous ligand that binds to an orphan opioid receptor offer approaches that may be taken to isolate the ligand for this receptor.

**1996 SUMMER NEUROPEPTIDE CONFERENCE
PARTICIPANTS**

Dr. Nouredine Brakch
Centre Hospitalier
Universitaire Vaudois
Avenue P. Decker
1011 Lausanne
SWITZERLAND
41-21-314-0741
FAX 41-21-3140761

Dr. Douglas Brenneman
Sect Devel Mol Pharmacology
NICHD, NIH
Building 49 Room 5A38
Bethesda, MD 20892
301-496-7649
FAX 301-480-5041

Dr. Robert Burrier
Schering Plough Pharmaceuticals
2015 Galloping Hill Road
Kenilworth, NJ 07033
908-298-3235
FAX 908-298-3294

Dr. James Cassella
Neurogen Corporation
35 Northeast Industrial Road
Branford, CT 06405
203-488-8201
FAX 203-488-3302

Dr. I. G. Charyeva
Dept. Morphology
Russia State Medical University
Ostrovityanova Str 1
117869 Moscow
RUSSIA
95-434-0037
FAX 95-434-4787

Dr. Linda Cornfield
Bayer Corporation
400 Morgan Avenue
West Haven, CT 06516
203-931-5456
FAX 203-937-2686

Dr. Jacqueline Crawley
Sect Behavioral Neuropharm, ETB
National Inst Mental Health
Building 10 Room 4D11
Bethesda, MD 20892-1375
301-496-7855
FAX 301-480-1164

Dr. Alejandro Daniels
Division of Pharmacology
Glaxo/Burroughs Wellcome Company
3030 Cornwallis Road
Research Triangle Park, NC 27709
919-483-1321
FAX 919-315-8890

Dr. Scott Dax
The R.W. Johnson Pharmaceutical
Research Institute
Welsh and McKean Roads
Spring House, PA 19477-0776
215-628-5211
215-628-4985

Dr. David de Wied
Rudolf Magnus Inst Neurosciences
Department of Med Pharmacology
Utrecht University
Universiteitsweg 100
3584 CG Utrecht
THE NETHERLANDS
31-30-2538846
FAX 31-30-2539032

Dr. Yvan Dumont
Douglas Hospital Research Ctr
McGill University
6875 Boulevard LaSalle
Verdun, Quebec H4H 1R3
CANADA
514-762-3048
FAX 514-762-3034

Dr. Jacques Epelbaum
Directeur de Recherche INSERM
Unite Dynamique Systemes Neuroendocriniens
INSERM U 159 Centre Paul Broca
2ter, rue d'Alesia
75014 Paris
FRANCE
33-1-4078-9222
33-1-4580-7293

Dr. Veronique Fardin
Neurochemistry Department
Rhône-Poulenc Rorer
13 quai Jules Guesde
94403 Vitry/Seine Cedex
FRANCE
33-1-4573-8068
FAX 33-1-4573-7653

Dr. Donald H. Ford
Council for Tobacco Research
900 Third Avenue
New York, NY 10022
212-421-8885

Dr. Jeffrey M. Friedman
Howard Hughes Med Res Inst
Rockefeller University
1230 York Avenue, Box 305
New York, NY 10021
212-327-8800
FAX 212-327-7420

Dr. Pierrette Gaudreau
Neuroendocrinology Lab
Notre-Dame Hospital Res Ctr
Room M-5226, 1560 East Sherbrooke St.
Montreal, Quebec H2L 4M1
CANADA
514-876-7159
FAX 514-876-6630

Dr. Donald R. Gehlert
Mail Code 0510
Lilly Research Labs
Eli Lilly Corporate Center
Indianapolis, IN 46285
317-276-1810
FAX 317-276-5546

Dr. Christophe Gerald
Synaptic Pharmaceutical Corp
215 College Road
Paramus, NJ 07652
201-261-1331
FAX 201-261-0623

Dr. Gregory E. Gonye
E.I. duPont de Nemours & Co.
Experimental Station
E328/B59
Wilmington, DE 19880
302-895-4139
FAX 302-695-8901

Dr. Illana Gozes
Dept. Clinical Biochemistry
Sackler School of Medicine
Tel Aviv University
Tel Aviv, ISRAEL
972-3-640-7240
FAX 972-640-8541

Dr. Eric Grouzmann
Centre Hospitalier
Universitaire Vaudois
Avenue P. Decker
1011 Lausanne
SWITZERLAND
41-21-314-0741
41-21-314-0761

Dr. Lawrence Grupp
Department of Pharmacology
University of Toronto
Med. Sci. Bldg. Room 4311
Toronto, Ontario M5S 1A8
CANADA
416-978-5155
FAX 416-978-6395

Dr. Glen Hanson
Dept. Pharmacol. Toxicol.
Coll. Pharmacy, Scaggs Hall 112
University of Utah
Salt Lake City, UT 84112
801-581-3174
FAX 801-585-5111

Dr. Vahram Haroutunian
Department of Psychiatry
Bronx VA Medical Center
130 W. Kingsbridge Road
New York, NY 10468
718-584-9000 X6082
FAX 718-365-9622

Dr. Markus Heilig
Magnus Huss Clinic
Karolinska Hospital
17176 Stockholm
SWEDEN
46-8-729-4859
FAX 46-8-326369

Dr. Louis B. Hersh
Chairman, Department of Biochemistry
Chandler Medical Center
University of Kentucky Sch. Med.
800 Rose Street
Lexington, KY 40536-0084
606-323-5549
FAX 606-323-1037

Dr. James Heym
Pfizer Central Research
Eastern Point Road
Groton, CT 06340
860-441-3534

Dr. Raymond Hill
Department of Pharmacology
Merck, Sharp & Dohme Res Labs
Neuroscience Research Centre
Terlings Park, Harlow
Essex CM20 2QR
ENGLAND
44-1-279-440168
FAX 44-1-279-440713

Dr. Michael Jasper
Gastrointestinal Unit
Jackson 802, GRJ 724
Massachusetts General Hospital
Boston, MA 02114
617-726-3768
FAX 617-724-3535

Dr. Lee Kaplan
Gastrointestinal Unit
GRJ 724
Massachusetts General Hospital
Fruit Street
Boston, MA 02114
617-726-3768
FAX 617-724-3535

Ms. Pamela Kent
11 Marie Curie
Vanier Bldg., Room 203
University of Ottawa
Ottawa, Ontario
CANADA K1N 6N5
613-562-5800 x4847

Dr. Jeffrey Kordower
Department of Neurological Sciences
Rush Presbyterian Medical Center
2242 West Harrison Street
Chicago, IL 60612
312-633-1550
FAX 312-633-1586

Dr. Jon Levine
Dept. Neurobiol. & Physiol.
Northwestern University
2153 Sheridan Road
Evanston, IL 60201
708-491-7180
FAX 708-491-5211

Dr. Walter Lichtensteiger
Institute of Pharmacology
University of Zurich
Winterthurerstrasse 190
CH-8057 Zurich
SWITZERLAND
41-1-257-5970
FAX 41-1-257-5708

Dr. David MacLean
Pfizer Central Research
Eastern Point Road
Groton, CT 06340
860-441-5953
FAX 860-441-5302

Dr. Saïde Madani
Institut National de Neurologie
1a Rabta 1007
TUNIS
216-1-223-368
FAX 216-570-070

Dr. David Malin
University of Houston Clear Lake
Box 237
2700 Bay Area Blvd.
Houston, TX 77058
713-283-3339
713-283-3405

Ms. Judith McIntosh
University of Ottawa
Department of Psychology
203-11 Marie Curie
Ottawa, Ontario
CANADA K1N 5N6
613-562-5800 X4847

Dr. Michael McDonald
Section on Behavioral Neuropharmacology
Experimental Therapeutics Branch
National Institute of Mental Health
Building 10 Room 4D11
Bethesda, MD 20892
301-496-4838
FAX 301-480-1164

Dr. Stafford McLean
Pfizer Central Research Division
Building 118, Room 217
Eastern Point Road
Groton, CT 06340
860-441-4563
FAX 860-441-1017

Dr. Paul Micevych
Department of Neurobiology
University of California
Health Sciences Center
Los Angeles, CA 90024
310-206-8265
FAX 310-825-2224

Dr. Zul Merali
Dept. Psychol. & Pharmacol.
University of Ottawa
11 Marie Curie
Ottawa ON K1N 6N5
CANADA
613-564-2969
FAX 613-564-9569

Dr. Louis Muglia
Div. of Endocrinology
Children's Hospital
Harvard Medical School
300 Longwood Avenue
Boston, MA 02115
617-355-7442
FAX 617-730-0741

Dr. Donald Pfaff
Rockefeller University
1230 York Avenue
New York, NY 10021
212-327-8666
FAX 212-327-8664

Dr. Thomas A. Pittler
Neurogen Corporation
35 NE Industrial Road
Branford, CT 06405
203-488-8201
FAX 203-481-8663

Dr. A. S. Pylaev
Head of Dept. of Morphology
Russia State Medical University
Ostrovityanova str., 1
117869 Moscow
RUSSIA
434-00-37

Dr. Remi Quirion
Director, Neuroscience Division
Douglas Hospital Research Centre
6875 Boulevard LaSalle
Verdun, Quebec H4H 1R3
CANADA
514-762-3048
FAX 514-762-3034

Dr. Regina Richter
Inst. Mol. Pharmacology
Alfred-Kowalke Str. 4
10315 Berlin
GERMANY
49-30-51551-237
FAX 49-30-5128-014

Dr. Ken Rohrbach
DuPont Merck Pharmaceutical Co.
Experimental Station, Bldg. 400
Wilmington, DE 19880-0400
302-695-7225
FAX 302-695-3730

Dr. James Rosamond
Astra Arcus USA
P.O. Box 20890
Rochester, NY 14602
716-274-5586
FAX 716-272-3931

Dr. William Rostene
INSERM U. 339
Hôpital Saint-Antoine
184, rue du Faubourg Saint-Antoine
75571 Paris CEDEX 12
FRANCE
33-1-4928-4676
FAX 33-1-4340-8270

Dr. Wayne Rowe
Douglas Hospital Research Center
6875 LaSalle Boulevard
Montreal, Quebec
CANADA H4H 1R3
514-762-3048
FAX 514-762-3034

Dr. Zoltan Sarnyai
Laboratory of Neuroendocrinology
The Rockefeller University
1230 York Avenue
New York, NY 10021
212-327-8625
FAX 212-327-8634

Dr. Lee Schechter
CNS Disorders, CN 8000
Wyeth-Ayerst Research
Princeton, NJ 08543-8000
980-274-4060
FAX 908-274-4020

Dr. Hans Schmitthenner
Astra Arcus USA
Department of Chemistry
Box 20890
Rochester, NY 14602
716-274-5589
FAX 716-272-3931

Dr. Kim Seroogy
Dept. Anatomy and Neurobiology
University of Kentucky
Chandler Medical Center
Lexington, KY 40536-0084
606-257-6038
FAX 606-323-5946

Dr. Claudine Serradeil-Le Gal
Sanofi Recherche
Centre de Toulouse
195 Route d'Espagne, B.P. 1169
31036 Toulouse Cedex
FRANCE
33-6116-2384
FAX 33-6116-2586

Dr. Roy G. Smith, Vice President
Merck Research Laboratories
P.O. Box 2000, R80K
Rahway, NJ 07065
908-594-7108
FAX 908-594-1250

Dr. Michael A. Statnick
Lilly Research Labs
Lilly Corporate Center
Indianapolis, IN 46285-0424
317-276-6856
FAX 317-276-9086

Dr. Franco Vaccarino
Clarke Institute of Psychiatry
250 College Street
Toronto, Ontario, M5T 1R8
CANADA
416-979-4675
FAX 416-979-4695

Dr. Mary Walker
Synaptic Pharmaceutical Corporation
215 College Road
Paramus, NJ 07652
201-261-1331
FAX 201-261-0623

Dr. Friedbert Weiss
Department of Neuropharmacology
The Scripps Research Institute
10666 North Torrey Pines Road
La Jolla, CA 92037
619-784-7068
FAX 619-784-7393

Dr. S. Wilson
SmithKline Beecham Pharmaceuticals
New Frontiers Science Park North
Third Avenue, Harlow, Essex
CM19 5AW ENGLAND
44-1-279-622598
FAX 44-1-279-622555

Richard E. Zigmond, Ph.D.
Department of Neuroscience
Case Western Reserve University
School of Medicine
10090 Euclid Avenue
Cleveland, OH 44106-4975
216-368-4614
FAX 216-368-4650

NOTES

NOTES

NOTES

NOTES